

WHAT IS CLAIMED IS:

1. A method for isolating heat shock protein receptor positive cells comprising:

- 5
- (a) contacting a solid phase containing heat shock protein with a population of cells comprising heat shock protein receptor positive cells, for a time period and under conditions sufficient to allow binding of the heat shock protein
- 10
- receptor positive cells to the solid phase;
- (b) removing the cells that are not bound to the solid phase; and
- 15
- (c) eluting the bound heat shock protein receptor positive cells from the solid phase.

2. A method for isolating heat shock protein receptor positive cells comprising:

20

3. A method for isolating heat shock protein receptor positive cells comprising the following steps:

- 25
- (a) contacting a population of cells comprising heat shock protein receptor positive cells with a heat shock protein comprising an affinity tag, for a time period and under conditions sufficient to allow binding of the heat shock protein to the heat shock protein receptor
- 30
- positive cells such that the heat shock protein receptor positive cells are labeled with the affinity tag;
- (b) contacting the population of cells with a solid phase containing a binding partner

of the affinity tag, for a time period and under conditions sufficient to allow binding of the labeled heat shock protein receptor positive cells to the solid phase;

(c) removing the cells that are not bound to the solid phase;

(d) eluting the labeled heat shock protein receptor positive cells from the solid phase.

4. A method for isolating a heat shock protein receptor positive cells comprising the following steps:

(a) contacting a population of cells comprising heat shock protein receptor positive cells with fluorescently labeled heat shock protein for a time period and under conditions sufficient to allow binding of the labeled heat shock protein to heat shock protein receptor positive cells such that the heat shock protein receptor positive cells are labeled fluorescently;

(b) separating the heat shock protein receptor positive cells that are fluorescently labeled from the unlabeled cells.

5. The method of claim 4 wherein the heat shock protein receptor positive cells are separated by fluorescent activated cell sorting.

6. The method of claim 2, 3, or 4 wherein the heat shock protein is gp96.

7. The method of claims 2, 3, or 4 wherein the heat shock protein is selected from the group consisting of heat shock protein 70, heat shock protein 90, heat shock protein 100 and an sm heat shock protein.

5

8. A population of cells substantially enriched for heat shock protein receptor positive cells.

9. A purified population of heat shock protein
10 receptor positive cells.

10. A population of cells substantially enriched for heat shock protein receptor positive cells prepared by the method of claims 1, 2, 3, or 4.

15

11. A purified population of heat shock protein receptor positive cells prepared by the method of claims 1, 2, 3, or 4.

20

12. The population of cells of claim 8, 9, 10, or 11 which are human cells.

13. A method for isolating a heat shock protein receptor comprising:

25

(a) preparing an extract of heat shock protein receptor positive cells;

(b) contacting a heat shock protein with the extract for a time period and under conditions sufficient for the heat shock protein receptor in the extract to bind the heat shock protein; and

30

(c) recovering the heat shock protein receptor bound to the heat shock protein.

14. The method of claim 13 wherein the heat shock protein comprises an affinity tag and wherein the method further comprises contacting the extract and the heat shock protein with a solid phase containing a binding partner of the affinity tag for a time period and under conditions sufficient to allow binding of the heat shock protein to the solid phase prior to step (c).

15. A method for isolating a heat shock protein receptor comprising:

- (a) preparing an extract of heat shock protein receptor positive cells;
- (b) contacting an antibody to heat shock protein receptor with the extract for a time period and under conditions sufficient for the heat shock protein receptor in the extract to bind to the antibody; and
- (c) recovering the bound heat shock protein receptor from the antibody.

16. The method of claim 10 wherein the antibody comprises an affinity tag and wherein the method further comprises prior to step (c) the step of contacting the antibody bound to heat shock protein receptor from the extract bound to heat shock protein receptor from the extract with a solid phase containing a binding partner of the affinity tag for a time period and under conditions sufficient to allow binding of the affinity tag to the binding partner on the solid phase prior to step (c).

17. A purified heat shock protein receptor which is isolated according to the method of claim 14, 15, or 16.

18. The heat shock protein receptor of claim 17 is a gp96 receptor.

19. The heat shock protein receptor of claim 17
5 selected from the group consisting of an Hsp70 receptor, an Hsp 90 receptor, an Hsp100 receptor, and an smHsp receptor.

20. The heat shock protein receptor of claim 17 is a human protein.

10

21. A method for preparing an antibody to a heat shock protein receptor comprising:

15

- (a) immunizing an animal with heat shock protein receptor or a immunogenic fragment thereof;
- (b) obtaining serum from the immunized animal;
- (c) screening the serum for the ability to bind or to heat shock protein receptor or antigenic fragment thereof, or to heat shock protein receptor positive cells, or to inhibit binding of heat shock protein to heat shock protein receptor positive cells;
- (e) recovering the antibody from the serum with said ability.

20

25

22. A method for preparing an antibody to a heat shock protein receptor comprising:

30

- (a) immunizing an animal with heat shock protein receptor or an immunogenic fragment thereof;
- (b) obtaining antibody-secreting cells from the immunized an animal;

- 5
- 10
- 15
- (c) fusing the antibody-secreting cells with an immortalized cell to produce hybridomas secreting monoclonal antibodies;
 - (d) screening the hybridomas for the ability of their secreted antibodies to bind to heat shock protein receptor positive cells, or to bind to heat shock protein receptor or antigenic fragment thereof, or to inhibit binding of heat shock protein to heat shock protein receptor positive cells;
 - (e) recovering the antibody secreted by a hybridoma with said ability.

23. A method for preparing an antibody to a heat shock protein receptor comprising:

- 20
- 25
- 30
- (a) immunizing an animal with heat shock protein receptor positive cells;
 - (b) obtaining serum from the immunized animal;
 - (c) screening the serum for the ability to bind to heat shock protein receptor positive cells or to inhibit binding of heat shock protein to heat shock protein receptor positive cells, or to bind to heat shock protein receptor or antigenic fragment thereof;
 - (e) recovering the antibody from the serum with said ability.

24. A method for preparing an antibody to a heat shock protein receptor comprising:

- 5 (a) immunizing an animal with heat shock protein receptor positive cells;
- (b) obtaining antibody-secreting cells from the immunized animal;
- 10 (c) fusing the antibody-secreting cells with a an immortalized cell to produce hybridomas secreting monoclonal antibodies;
- (d) screening the hybridomas for the ability of their secreted antibodies to bind to heat shock protein receptor positive cells or to inhibit binding of heat shock protein to heat shock protein receptor positive cells or to bind to heat shock protein receptor or antigenic fragment thereof;
- 15 (e) recovering the antibody secreted by a hybridoma with said ability.

20 25. An antibody to the protein of claim 17.

26 The antibody of claim 22 or 24 which is monoclonal.

25 27. The antibody of claim 21 or 23 which is polyclonal.

28. A method for isolating a nucleic acid molecule encoding heat shock protein receptor comprising the following steps:

30

- (a) contacting a labeled nucleic acid probe with recombinant DNA molecules derived from recombinant cells containing a plurality of DNA molecules from heat

shock protein receptor positive cells,
for a time period and under conditions
sufficient to allow hybridization of the
labeled probe to the DNA molecules,
wherein the labeled probe has a nucleic
acid sequence that comprises a sequence
that encodes heat shock protein receptor
or an antigenic fragment thereof;

- (b) identifying the recombinant cell
containing the DNA molecule to which the
labeled probe hybridized;
- (c) recovering the DNA molecule present in
the recombinant cell.

29. The method of claim 24 further comprising:

- (d) repeating steps (a) through (c) wherein
the recombinant cells are the cells of
step (b), until one DNA molecule is
recovered from step (c).

30. The method of claim 24 wherein the plurality
of DNA molecules have been selected by subtractive
hybridization for DNA molecules that are expressed in heat
shock protein receptor positive cells but not in heat shock
protein receptor negative cells.

31. The method of claim 27 wherein the heat shock
protein receptor positive cells are human cells.

32. A method for isolating a cDNA molecule
encoding heat shock protein receptor comprising:

- (a) contacting a solid phase containing an
antibody to heat shock protein receptor
with recombinant cells expressing the

proteins encoded by a plurality of cDNA molecules synthesized from heat shock protein receptor positive cells, for a time period and under conditions sufficient to allow binding of the recombinant cells to the antibody on the solid phase;

- (b) removing the recombinant cells that are not bound to the solid phase;
- (c) eluting the bound recombinant cells from the solid phase; and
- (d) recovering the cDNA molecule present in the recombinant cells.

33. The method of claim 28, further comprising:

- (e) replicating the recovered cDNA molecules;
- (f) introducing the cDNA molecule into cells capable of expressing the proteins encoded by the cDNA molecule; and
- (g) repeating steps (a) through (d) wherein the recombinant cells are the cells resulting from step (f), until one cDNA molecule is recovered from step (d).

34. A method for isolating a cDNA molecule encoding heat shock protein receptor comprising:

- (a) contacting a solid phase containing heat shock protein with recombinant cells expressing the proteins encoded by a plurality of cDNA molecules synthesized from heat shock protein receptor positive cells, for a time period and under conditions sufficient to allow binding of the recombinant cells to the solid phase;

- (b) removing the recombinant cells that are not bound to the solid phase;
(c) eluting the bound recombinant cells from the solid phase; and
5 (d) recovering the cDNA molecule present in the recombinant cells.

35. The method of claim 30, further comprising:

- (e) replicating the recovered cDNA molecules;
10 (f) introducing the cDNA molecule into cells capable of expressing the proteins encoded by the cDNA molecule; and
(g) repeating steps (a) through (d) wherein the recombinant cells are the cells
15 resulting from step (f), until one cDNA molecule is recovered from step (d).

36. The method of claim 30 wherein the solid phase is a magnetic bead.
20

37. The method of claim 30 wherein the plurality of cDNA molecules have been selected by subtractive hybridization for cDNA molecules that are expressed in heat shock protein receptor positive cells but not in heat shock
25 protein receptor negative cells.

38. A method for isolating a cDNA molecule encoding heat shock protein receptor comprising:

- (a) contacting recombinant cells expressing the proteins encoded by a plurality of
30 cDNA molecules synthesized from heat shock protein receptor positive cells on a solid phase with a labeled heat shock protein, for a time period and under

conditions sufficient to allow binding of the labeled heat shock protein to the recombinant cells;

(b) removing the labeled heat shock protein that is not bound to the recombinant cells;

(c) eluting the recombinant cells to which the labeled heat shock protein is bound from the solid phase;

(d) recovering the cDNA molecule present in the recombinant cells.

39. The method of claim 34 further comprising:

(e) replicating the recovered cDNA molecules;

(f) introducing the cDNA molecule into cells capable of expressing the proteins encoded by the cDNA molecule; and

(g) repeating steps (a) through (d) wherein the recombinant cells are the cells resulting from step (f), until one cDNA molecule is recovered from step (d).

40. A method for isolating a cDNA molecule encoding heat shock protein receptor comprising:

(a) contacting recombinant cells expressing the proteins encoded by a plurality of cDNA molecules synthesized from heat shock protein receptor positive cells on a solid phase with an antibody to heat shock protein receptor, for a time period and under conditions sufficient to allow binding of the antibody to the recombinant cells;

- 5
- (b) removing the antibody that is not bound to the recombinant cells;
 - (c) eluting the recombinant cells to which the antibody to is bound from the solid phase; and
 - (d) recovering the cDNA molecule present in the recombinant cells.

10 41. The method of claim 34 further comprising:

- 15
- (e) replicating the recovered cDNA molecules;
 - (f) introducing the cDNA molecule into cells capable of expressing the proteins encoded by the cDNA molecule; and
 - (g) repeating steps (a) through (d) wherein the recombinant cells are the cells of step (f), until one cDNA molecule is recovered from step (d).

20 42. A method for isolating a cDNA molecule encoding heat shock protein receptor comprising the following steps:

- 25
- (a) contacting recombinant cells expressing the proteins encoded by a plurality of cDNA molecules synthesized from heat shock protein receptor positive cells with a heat shock protein comprising an affinity tag, for a time period and under conditions sufficient to allow binding of the heat shock protein to the recombinant cells such that the recombinant cells are labelled with the affinity tag;
 - (b) contacting the recombinant cells with a solid phase containing a binding partner of the affinity tag, for a time period
- 30

and under conditions sufficient to allow binding of the labeled recombinant cells to the binding partner on the solid phase;

- 5 (c) removing the recombinant cells that are not bound to the solid phase;
- (d) eluting the labeled recombinant cells from the solid phase; and
- 10 (e) recovering the cDNA molecule present in the labeled recombinant cells.

43. A method for isolating a cDNA molecule encoding heat shock protein receptor comprising the following steps:

- 15 (a) contacting a solid phase containing an antibody to heat shock protein with recombinant cells expressing the proteins encoded by a plurality of cDNA molecules synthesized from heat shock protein
- 20 receptor positive cells, for a time period and under conditions sufficient to allow binding of the recombinant cells to the antibody on the solid phase;
- (b) removing the recombinant cells that are
- 25 not bound to the solid phase;
- (c) eluting the bound recombinant cells from the solid phase; and
- (d) recovering the cDNA molecule present in
- 30 the recombinant cells.

44. The method of claim 39 further comprising:
- (e) replicating the recovered cDNA molecules;

5

- (f) introducing the cDNA molecule into cells capable of expressing the proteins encoded by the cDNA molecule; and
- (g) repeating steps (a) through (d) wherein the recombinant cells are the cells of step (f), until one cDNA molecule is recovered from step (d).

45. The method of claim 39 wherein the solid phase
10 is a magnetic bead.

46. The method of claim 39 wherein the plurality of cDNA molecules have been selected by subtractive hybridization for cDNA molecules that are expressed in heat
15 shock protein receptor positive cells but not in heat shock protein receptor negative cells.

47. The method of claim 35, 37, 39, or 40 wherein the heat shock protein receptor positive cells are human
20 cells.

48. An isolated nucleic acid molecule which is isolated according to the method of claim 35, 37, 39, or 40 which encodes a receptor for heat shock protein.
25

49. The isolated nucleic acid molecule of claim 45 is of human origin.

50. A method for preparing recombinant heat shock
30 protein receptor (HSPR) comprising:

- (a) culturing cells containing a recombinant nucleic acid molecule comprising a sequence encoding heat shock protein receptor operably linked to a promoter,

such that the heat shock protein receptor is expressed by the cells; and

- (b) recovering the expressed heat shock protein receptor.

5

Sub 1

51. A method for screening a molecule for activity to modulate heat shock protein receptor levels or activity comprising:

10

- (a) contacting cells with the molecule; and
(b) comparing the level of heat shock protein receptor protein, mRNA or activity in cells contacted with the molecule to the amount of heat shock protein receptor protein, mRNA, or activity, in cells not so contacted,

15

wherein an increase or decrease in the amount of heat shock protein receptor protein, mRNA, or activity in the contacted cells relative to the amount of heat shock protein receptor protein, mRNA, or activity in the cells not so contacted indicates that the molecule has activity to modulate heat shock protein receptor levels or activity.

20

52. A method for screening a molecule for activity to modulate, directly or indirectly, the ability of heat shock receptor positive cells to stimulate the activation of cytotoxic T cells in vitro comprising:

25

- (a) adding the molecule to a mixture of heat shock protein receptor positive cells and cytotoxic T cells under conditions conducive to the activation of cytotoxic T cells; and
(b) comparing antigenic cell cytotoxicity of said T cells with the cytotoxicity of T

30

cells that are formed in the absence of
said molecule,
wherein a lower or higher degree of
cytotoxicity indicates that the molecule modulates the
5 activation of cytotoxic T cells.

add
add
10
add
ADD
D7
ADD
F7
add 911

15

20

25

30